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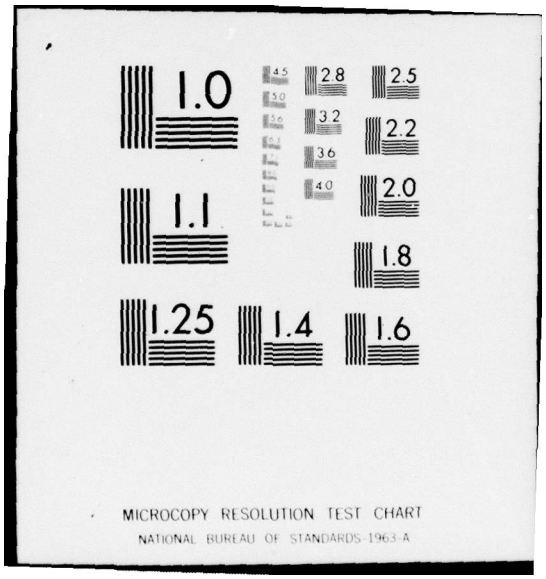
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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)

Electroimmunodiffusion Studies of α Chain, Secretory Piece and Secretory IgA.

5. TYPE OF REPORT & PERIOD COVERED
manuscript for publication
March 74 - 1977

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)

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8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS

U.S. Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, D. C. 20012

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
61102A 3A161102b71R
Task 04 OF6024 Work Unit 065

11. CONTROLLING OFFICE NAME AND ADDRESS

U.S. Army Med. Research & Development Command,
HQDA (SGRD-RP)
Washington, D.C. 20314

12. REPORT DATE
15 March 1977

13. NUMBER OF PAGES
14

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)

15. SECURITY CLASS. (of this report)
unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Secretory IgA, electroimmunodiffusion, application for biological fluids.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Commercially available antisera to secretory piece, colostrum, and α chain were evaluated for use in electroimmunodiffusion of secretory IgA and secretory piece in biological fluids. Quantitation of secretory IgA presents difficulty since antisera to α chain indiscriminately precipitates both serum IgA and secretory IgA (11S) and production of antisera specific for antigenic determinants on secretory IgA is laborious and expensive. Problems in using currently available commercial antisera to colostrum and free secretory piece arise because they are multispecific; however, a technique modification described here overcomes that

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difficulty.

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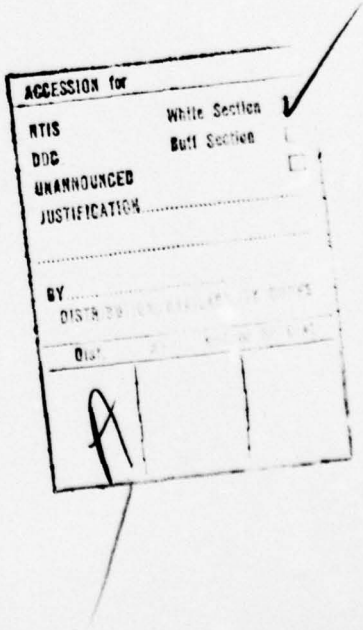
The high level sensitivity of EID allows quantative assay of proteins in biological fluids collected in μl amounts, thus eliminating the need for stimulation of secretions and sample concentration.

Military Justification

Protection of mucosal surfaces of the gastrointestinal, respiratory and urogenital tracts by immunoglobulins, particularly IgA, is of utmost importance in preventing serious infectious diseases, many of which are acquired in tropical and other foreign countries. Military personnel in these potential combat areas are especially susceptible to microbial infections which affect nasopharyngeal and gastrointestinal surfaces. The oral cavity offers the most easily accessible secretory IgA - mucous membrane system for study of the agents important in maintaining the integrity of the mucosal surfaces.

Research oriented toward the development of vaccines requires improved methodology for determination of the immune response by quantitation of antibodies produced following vaccination.

This study marks an advance in methodology for rapid easily performed quantitation and differentiation of secretory IgA and free secretory piece in saliva and other biological fluids, and therefore, is a valuable contribution to the mission oriented research dealing with the development of protective and preventive measures against infectious hazards encountered by a soldier throughout the world.



Electroimmunodiffusion Studies of α Chain, Secretory Piece and Secretory IgA

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Running head: Electroimmunodiffusion of IgA and Secretory Piece

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ABSTRACT

Commercially available antisera to secretory piece, colostrum, and α chain were evaluated for use in electroimmunodiffusion of secretory IgA and secretory piece in biological fluids. Quantitation of secretory IgA presents difficulty since antisera to α chain indiscriminately precipitates both serum IgA and secretory IgA (11S) and production of antisera specific for antigenic determinants on secretory IgA is laborious and expensive. Problems in using currently available commercial antisera to colostrum and free secretory piece arise because they are multispecific; however, a technique described here overcomes that difficulty.

Although monospecific antisera to I or P determinant would differentiate secretory piece or secretory IgA with one rocket precipitate, that same precipitate is identifiable in a multi-precipitate pattern by using a pure reference standard for establishment of a line of identity.

The high level sensitivity of EID allows quantative assay of proteins in biological fluids collected in µl amounts, thus eliminating the need for stimulation of secretions and sample concentration.

Electroimmunodiffusion (EID) has been shown to be an excellent method for quantitation of serum immunoglobulins in dilute solutions (2, 6, 14-16, 18).

In the EID technique electrophoresis of antigen into a monospecific antiserum-containing gel results in an antigen-antibody precipitate which possesses at equivalence a characteristic rocket shape, the height of which is related to the concentration of antigen. This technique has been found to be particularly useful for determining levels of immunoglobulins in unconcentrated saliva. The main immunoglobulin in saliva and other external secretions is secretory IgA (S-IgA) which is composed of an IgA dimer bound to a glycoprotein of epithelial origin termed secretory piece (S-P) (21). Unbound free S-P is commonly found in secretions devoid of S-IgA as well as in secretions containing S-IgA (3, 19). Brandtzaeg (4) and Brandtzaeg et al. (5) detected antigenic determinants on S-IgA (11S) which are absent on serum IgA (7S), and also found that free S-P possesses an I determinant which is inaccessible when bound to the S-IgA dimer. An additional antigenic determinant on S-IgA, J chain, has been described by Halpern and Koshland (9).

This report describes methodology for electroimmunodiffusion of serum IgA (7S), secretory IgA (11S), and free secretory piece in dilute biological secretions and evaluates

the potential of commercial antisera for use in quantitation of these proteins.

MATERIALS AND METHODS

Antisera. Rabbit antisera to human α chain, colostrum and free secretory piece were used throughout this study (Behring Diagnostics, Somerville, N.J.). According to the manufacturer antiserum to free secretory piece was produced by immunization with a purified S-P antigen and subsequent absorption with serum IgA and lactoferrin. Antiserum to human colostrum was produced by immunization with whole colostrum and absorption with human serum until no immunoprecipitation with human serum was observed upon immunoelectrophoresis or double diffusion (Personal communication, N. Rangoonwala, Behring Diagnostics).

Colostrum and parotid fluid. Secretory IgA (11S) was purified from colostrum by the method of Brandtzaeg et al. (5). Parotid fluid from a patient with hypogammaglobulinemia (provided by E. C. Tramont, Walter Reed Army Institute of Research, Washington, D.C.) and from a patient on dilantin therapy with complete IgA suppression (8) was the source of free secretory piece. Absence of IgA in these fluids was determined by immunoelectrophoresis and EID using antiserum to α chain. Parotid fluid was collected using Curby cups (7) placed over Stensen's duct. Human colostrum was collected up to three days postpartum. All specimens were stored at -70 C until used.

EID methodology. Electroimmunodiffusion was performed using either Indubiose 45 agarose (Fisher Scientific, Silver

Spring, Md.) or Behring agarose (Behring Diagnostics, Somerville, N.J.). One percent suspensions were heated to boiling in barbital buffer prepared as described by Axelson et al. (2), cooled to 55 C and thoroughly mixed with appropriate amounts of antiserum.

Clean 10 x 7 cm glass slides (Eastman Kodak Co., Rochester, N.Y.) placed on a leveling table, were layered with 10 ml of the agarose-antiserum mixture and cooled for 5 minutes at 4 C. Two mm diameter wells were then cut into the solidified gel with a well puncher (Bio Rad Laboratories, Richmond, Calif.) Slides were used immediately after preparation and were placed into an electrophoresis chamber containing the barbital buffer described above at 4 C with the wells adjacent to the cathode. Filter paper (Number 3, Whatman Inc., Clifton, N.J.) cut to fit the plates served as wicks.

Antigen samples (5 $\mu\ell$) were applied with a capillary (Ziptrol, Drummond Scientific Co., Broomall, Pa.) and electrophoresed through the gel (Table 1). Following electrophoresis the slides were washed for 10 min 0.14M NaCl, rinsed in distilled water, carefully covered with filter paper, pressed, dried, and stained for 1 min in a 5% solution of Coomassie Brilliant Blue R-250 (Eastman Kodak, Rochester, N.Y:) in ethanol - glacial acetic acid - water (45:10:45). Destaining was accomplished in the dye-free solvent.

Identification of precipitates by EID reactions of identity was performed by modification of techniques previously described (1, 13, 20).

RESULTS

Effects of antiserum concentration, voltage, time, and agarose on EID. Antiserum concentrations in agarose, electrophoresis voltage, and duration for optimal electroimmunodiffusion of salivary levels of α chain, secretory IgA, and free secretory piece using Behring agarose are summarized in Table 1. Staining of the precipitates after electrophoresis was required for visualization, however, carbamylation of specimens or antisera was not required to give clear visualization of precipitates.

The type of agarose used influenced both the height and clarity of rocket formation. EID in Indubiose 45 agarose resulted in rocket formation twice as high as in Behring agarose although clarity was greatly reduced. Visualization was improved, however, when the antiserum concentration was increased. Behring agarose was found to give the sharpest precipitate with the least concentration of antiserum and was therefore used throughout these studies.

α chain antiserum specificity. Behring anti-α chain was found to be monospecific in EID giving one rocket precipitate for all specimens tested including saliva, colostrum, purified secretory IgA and serum (Fig. 1). As expected no precipitate occurred with saliva from IgA deficient individuals.

Colostral antiserum specificity. Anti-colostrum interactions with whole saliva, IgA deficient saliva and secretory IgA produced EID patterns consisting of double rockets (Fig. 2b, d, and f). Complete absorption of α and light chain activity from colostral antiserum with

serum was attained as evidenced by the lack of precipitation with serum (Fig. 2a).

EID of concentrated parotid fluid and colostrum yielded triple rockets (Fig. 2c and e). Additional experiments have shown that by increasing the colostral antiserum concentration in the agarose two more rockets to colostrum were detectable. Indications that one of the major specificities of colostral antiserum was to a determinant on secretory IgA was demonstrated by a darkly staining rocket to secretory IgA (Fig. 2f).

Free secretory piece antiserum specificity. Precipitation patterns with free S-P antiserum in the agarose consisted of two rockets with whole saliva, parotid fluid, colostrum, and secretory IgA (Fig. 3b, c, e and f).

Only one precipitate occurred with IgA deficient saliva while no reaction occurred with serum (Fig. 3d and a).

The reaction of identity. Precipitin peaks (rockets) will fuse when immunologically identical antigens are electrophoresed from closely punched adjacent wells. This is shown in Fig. 4, where a line of identity was obtained between whole saliva and IgA deficient saliva in agarose containing antiserum to free secretory piece that was not monospecific.

DISCUSSION

Although commercial antiserum to α chain was found monospecific, the indiscriminate precipitation of both IgA (7S) and S-IgA (11S) precludes its use in identification of these two forms of IgA in biological fluids. Commercial colostral antiserum precipitated S-IgA but not serum IgA confirming specificity to an antigenic determinant on S-IgA other than α chain or light chains. The multiple precipitates resulting from EID of colostrum with colostral antiserum in the agarose indicates its ability to detect several antigens in biological fluids not found in serum, but complicates its use in EID making necessary a reference antigen for precipitate identification.

Free secretory piece is invariably present in saliva of IgA deficient individuals (19, 21). The EID pattern for IgA deficient saliva from two such individuals yielded a single rocket with antiserum to S-P demonstrating specificity for a determinant on the free S-P molecule. Possible presence of antibody to α chain was tested for by using serum IgA as antigen. No activity to α chain was found although the antiserum was not monospecific since IgA-containing specimens revealed a second precipitate. It was concluded that there were unabsorbed traces of antibody to an antigenic determinant on the S-IgA molecule. This conclusion is supported by the fact that only one precipitate occurs with IgA deficient saliva from two different individuals who otherwise would be expected to have salivary proteins similar to normal persons.

Although a monospecific antiserum to I or P determinants (4, 5) on S-IgA would identify S-P and S-IgA with one rocket precipitate, these same rockets are identifiable in a multi-precipitate pattern by utilizing a pure reference antigen for establishment of a line of idenity. This is illustrated in Fig. 4 where IgA deficient saliva served as a pure antigen to qualitatively identify precipitation due to free secretory piece. Such a line of identity is obtained by inclusion of a reference antigen in a well closely adjacent to the well containing the specimen to be assayed.

Fusion of precipitin lines will occur with peaks of immunologically identical antigens. The criteria used to establish immunological identity were total fusion of precipitates and identical precipitate morphology.

Interference by J chain antigen was not considered applicable to this assay since antiserum to J chain does not precipitate Ig polymers (11, 12, 17). Titers to J chain are at best low even when purified antigen is used. Furthermore, since J chain is hidden in the S-IgA dimer, probably due to a masking effect of secretory component (10, 11), it is unlikely there is antibody directed against it.

Our observations lead us to conclude that the presence of multiple precipitates in EID due to multispecific antisera does not preclude its use in qualitatively differentiating proteins and subsequently quantitating them if a known reference antigen is used in the assay.

TABLE 1. Electroimmunodiffusion of 7S IgA, Free Secretory Piece and 11S Secretory IgA in Saliva Using Commercial Antisera

Antiserum	Antiserum concentration in agarose[a]	Electrophoresis time (hrs)	Voltage applied (volts)
Alpha chain	0.5 - 0.7%	3½ - 4[b]	45.0 9 volts/cm[c]
Free secretory piece	0.1 - 1.5%	2 - 3	25.0 5 volts/cm
Colostrum	0.75 - 1%	2½	35.0 7 volts/cm

a Lack of significant titer variation between Behring antisera batches allows consistency in concentration of antisera in agarose.

b Fresh buffer supplied after 2-2½ hours.

c Volts/cm of distance between wick-agarose interface.

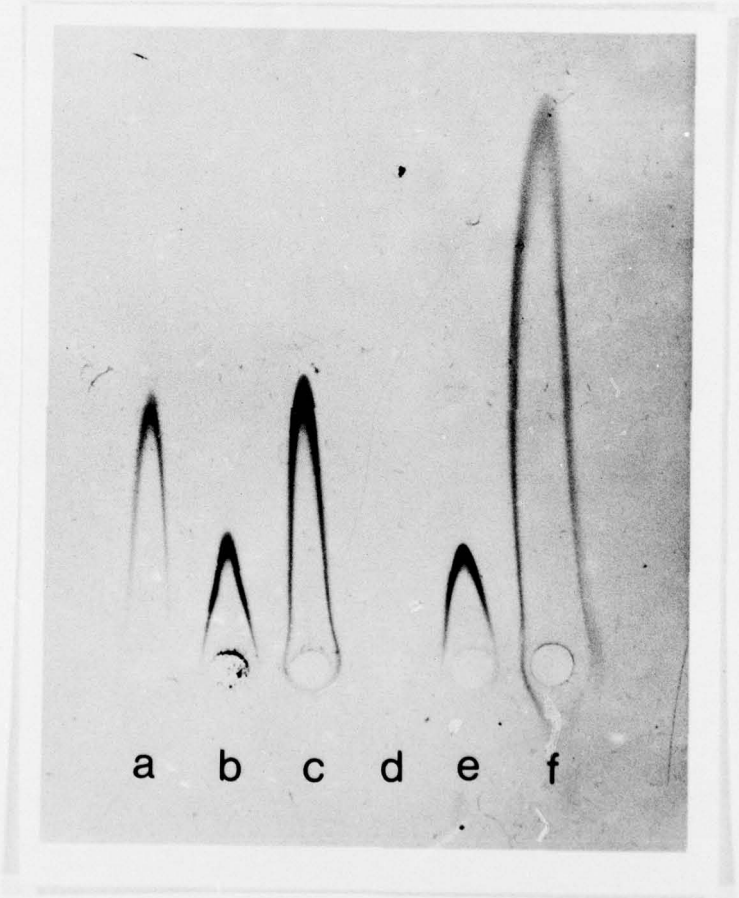

Fig. 1. Electroimmunodiffusion of (a) serum (diluted 1/100); (b) whole saliva; (c) parotid fluid (concentrated 4x); (d) IgA deficient saliva; (e) defatted colostrum (diluted 1/10); and (f) purified secretory IgA in agarose containing antiserum to α chain.

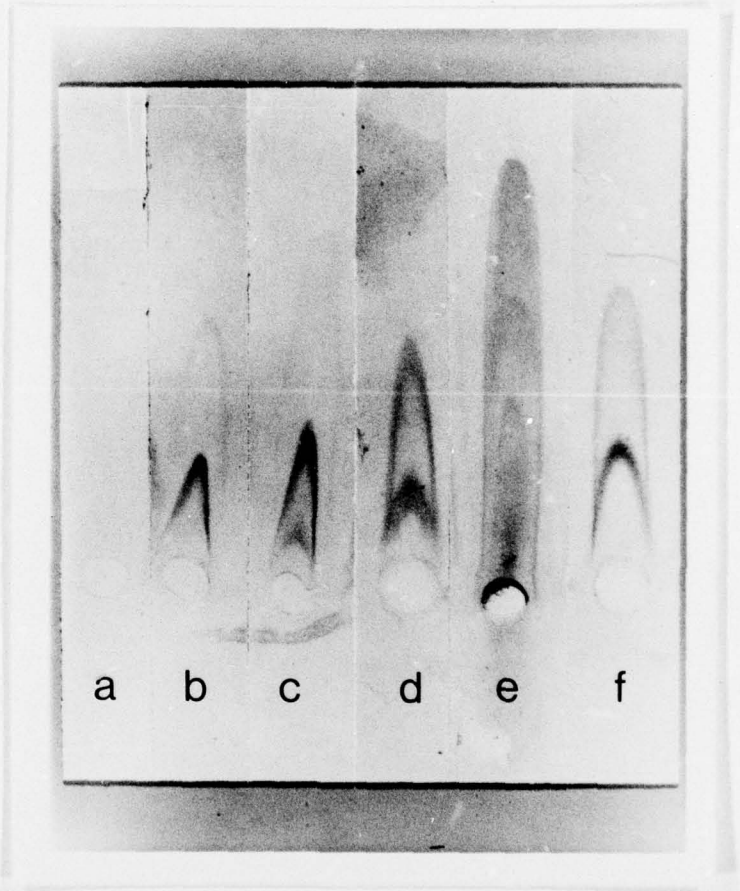

Fig. 2. Electroimmunodiffusion of (a) serum, (b) whole saliva, (c) parotid fluid, (d) IgA deficient saliva, (e) colostrum and (f) purified secretory IgA in agarose containing antiserum to colostrum. A double rocket with purified secretory IgA reveals the presence of a contaminating antigen.

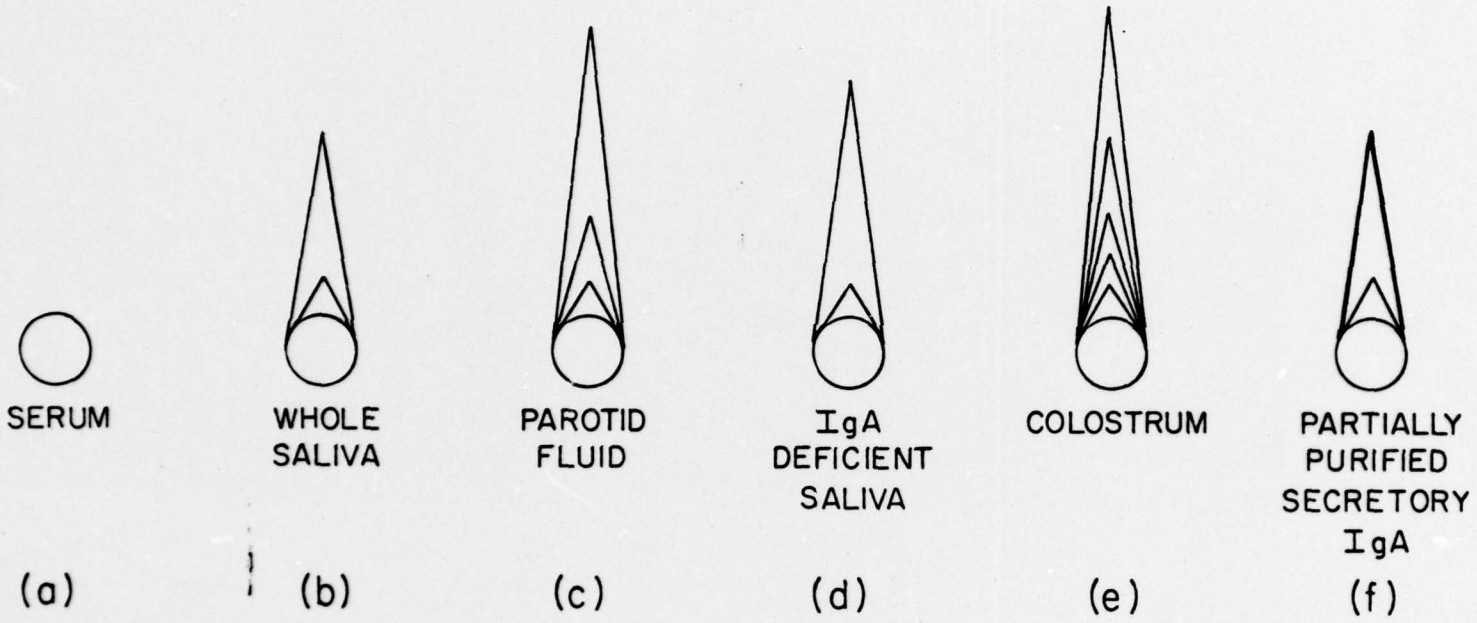

FIG. PRECIPITATION PATTERNS FROM ELECTROIMMUNODIFFUSION WITH COLOSTRAL ANTISERUM IN THE AGAROSE.

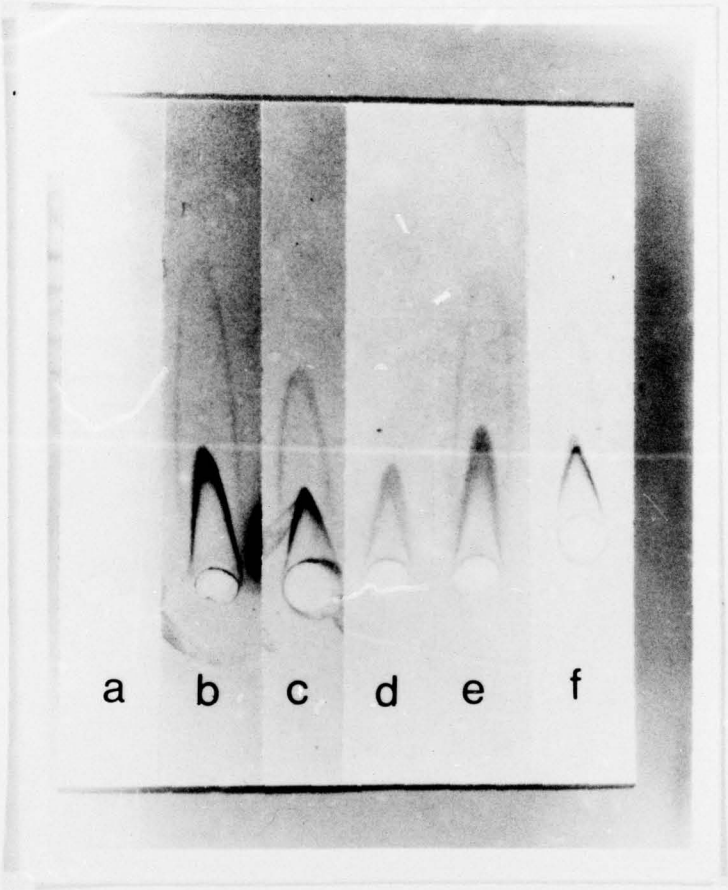

Fig. 3. Electroimmunodiffusion of (a) serum, (b) whole saliva, (c) parotid fluid, (d) IgA deficient saliva, (e) colostrum and (f) purified secretory IgA in agarose containing antiserum to free secretory piece.

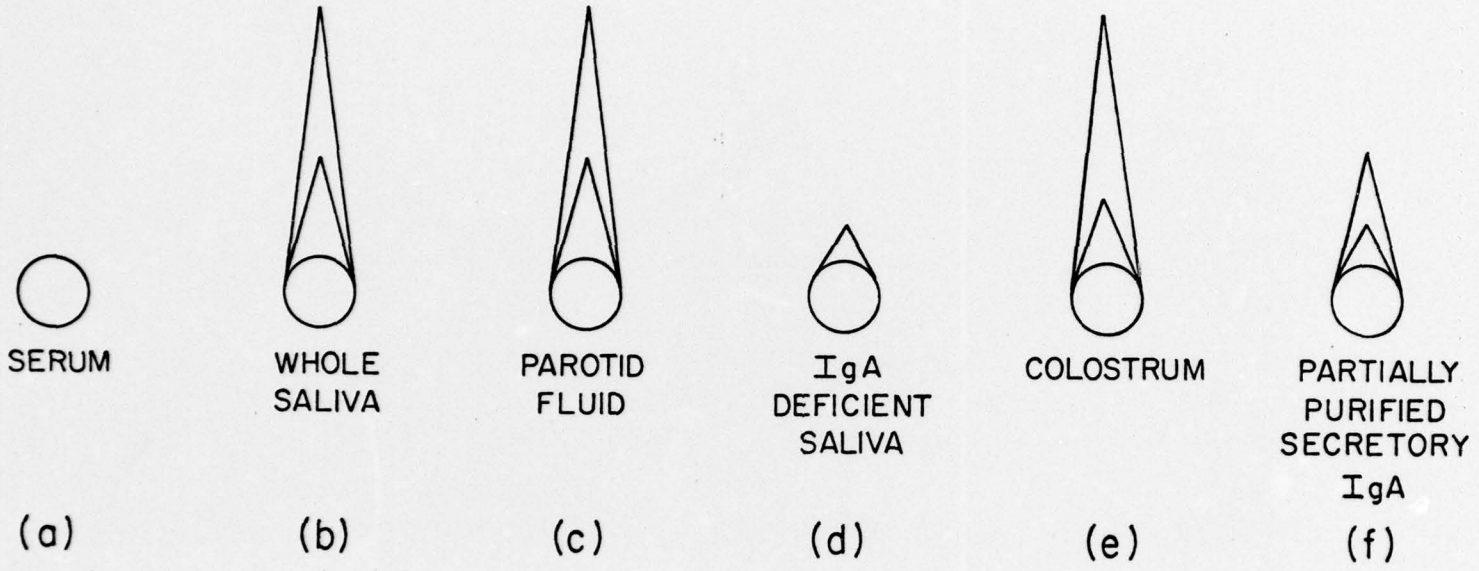

FIG PRECIPITATION PATTERNS FROM ELECTROIMMUNODIFFUSION WITH FREE SECRETORY PIECE ANTISERUM IN THE AGAROSE.

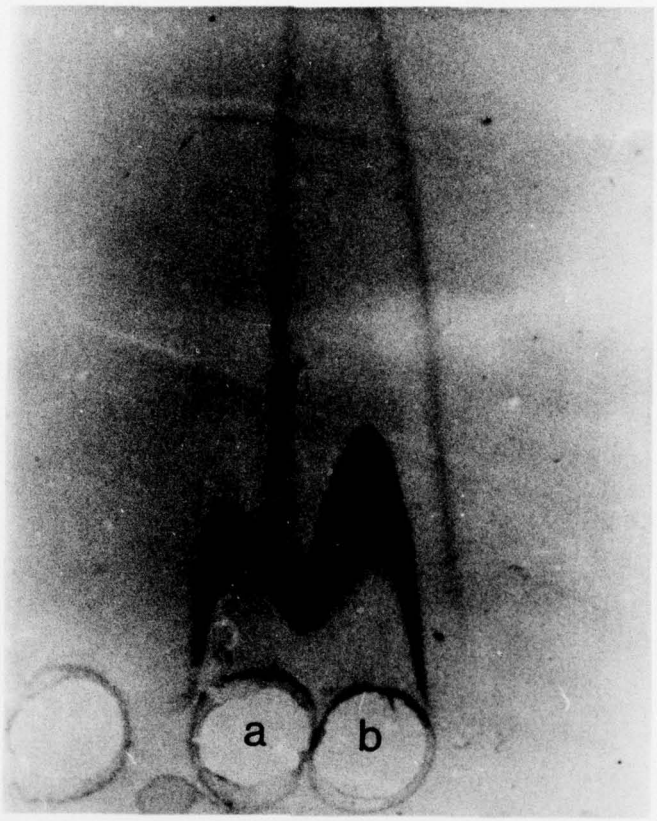

Fig. 4. Line of identity in agarose containing antiserum to free secretory piece between (a) whole saliva and (b) IgA deficient saliva. Variation in specificity between batches necessitates individual batch evaluation.

LITERATURE CITED

1. Axelsen, N.H., E. Bock, and J. Kroll. 1973. Comparison of antigens: the reaction of identity. Scand. J. Immunol. (suppl) 1:91-94.

2. Axelsen, N.H., B. Kroll, and B. Weeke. 1973. A manual of quantitative immunoelectrophoresis methods and applications. Scand. J. Immunol. (suppl) 1:1-67.

3. Brandtzaeg, P. 1971. Human secretory immunoglobulins. II Salivary secretions from individuals with selectively excessive or defective synthesis of serum immunoglobulins. Clin. Exp. Immunol. 8:69-85.

4. Brandtzaeg, P. 1971. Human serum immunoglobulins. IV Quantitation of free secretory piece. Acta. path microbiol. Scand. (section B) 79:189-203.

5. Brandtzaeg, P., I. Fjellanger, and S.T. Gjeruldsen. 1970. Human secretory immunoglobulins. I Salivary secretions from individuals with normal or low levels of serum immunoglobulins. Scand. J. Hematol (suppl) 12:1-85.

6. Crowle, A.J. 1973. Immunodiffusion, p. 353-402, 2nd ed. Academic Press, New York, N.Y.

7. Curby, W.A. 1953. Device for collection of human parotid saliva. J. Lab. Clin. Med. 41:493.

8. Grob, P.J., and G.E. Herold. 1972. Immunological abnormalities and hydantoins. Brit. Med. J. 2:561-563.

9. Halpern, M.S., and M.E. Koshland. 1970. Novel subunit in secretory IgA. Nature (London) 228:1276-1278.

10. Heremans, J.H. 1973. The IgA system in connection with local and systemic immunity, p. 3-22. In A.R. Lawton (ed.), Advances in experimental medicine and biology vol. 45. Plenum Press, New York.

11. Kobayashi, K., J.P. Vaerman, and H.H. Heremans. 1973. J chain determinants in polymeric immunoglobulins. Europ. J. Immunol. 3:185-191.

12. Kownatzki, E. 1973. Antigenic properties of J chain. J. Immun. Forsch. 145-333.

13. Kroll, J. 1969. Immunochemical identification of specific precipitin lines in quantitative immunoelectrophoresis patterns. Scand. J. Clin Lab. Invest. 24:55-60.

14. Laurell, C.B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. 15:45-52.

15. Lopez, M., T. Tsu, and E. Hyslap, Jr. 1969. Studies on electo-immunodiffusion: Immunochemical quantitation of proteins in dilute solutions. Immunochem. 6:513-526.

16. McClelland, D.B., N.D. Finlayson, R.R. Samson, I.M. Nairn, and D.J. Sherman. 1971. Quantitation of immunoglobulins in gastric juice by electroimmunodiffusion. Gastroent. 60:509-514.

17. Meinke, G.C., and H.L. Spiegelberg. 1974. Antigenic studies of J chain. J. Immunol. 112:1401-1406.

18. Merrill, D., T.F. Hartley, and H.N. Claman. 1967. Electro-immunodiffusion: A simple, rapid method for quantitation of immunoglobulins in dilute biological fluids. J. Lab. Clin. Med. 69:151-159.

19. South, M.A., M.A. Cooper, F.A. Wollheim, R. Hong, and R.A. Good. 1966. The IgA system: I. Studies of the transport and immunochemistry of IgA in the saliva. J. Exp. Med. 123:615-627.

20. Sweet, G.H., D.E. Wilson, and J.D. Gerber. 1973. Application of electroimmunodiffusion and crossed electroimmunodiffusion to the comparative serology of a microorganism (Histoplasma capsulatum). J. Immunol. 111:554-564.

21. Tomasi, T.B., Jr. and H.M. Grey. 1972. Structure and function of IgA. Prog. Allergy. 16:81-213.